increased the accumulation of the nucleotide in all tissues tested, apparently by inhibiting the hydrolysis of the compound to 5′-AMP by a very active diesterase which was also inhibited by theophylline.

Thus the glycogenolytic action of epinephrine in liver, heart, and skeletal muscle appears to be a result of the following sequence of events: epinephrine interacts with some insoluble component of the cell, giving rise to an increased accumulation of 3,5-AMP, which, in turn, interacts with more dispersed material, resulting in an increased conversion of inactive to active phosphorylase. The relation of one or more of the events in this sequence to other effects of epinephrine has not been clarified. It is interesting to note that particulate fractions from dog heart were most sensitive to isopropylnorepinephrine, less but equally sensitive to *l*-epinephrine and *l*-norepinephrine, and even less sensitive to *d*-epinephrine, as judged by the ability of these agents to increase 3,5-AMP accumulation. Furthermore, the heart phosphodiesterase which hydrolyzes and inactivates 3,5-AMP is even more sensitive to theophylline than to caffeine.

DISCUSSION

ACTIONS OF SEROTONIN AND EPINEPHRINE ON INTACT AND BROKEN CELL PREPARATIONS FROM THE LIVER FLUKE, *FASCIOLA HEPATICA*

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I would like to discuss briefly some aspects of the effects of epinephrine and of serotonin on the carbohydrate metabolism of a trematode parasite, the liver fluke, *Fasciola hepatica*. This organism metabolizes carbohydrate at a high rate. Production of propionic and acetic acids in an approximate ratio of 3:1 accounts for almost all of the carbohydrate utilized anaerobically. Only 4 to 8% of the metabolized carbohydrate is converted to lactic acid. Contrary to its effect on higher organisms, neither epinephrine nor norepinephrine has any action on the carbohydrate metabolism of these trematodes. On the other hand, serotonin and other indolalkylamines at low concentrations cause an increase in glucose utilization, glycogen breakdown and lactic acid production (1). Production of volatile fatty acids is not affected to a significant degree by serotonin. Since serotonin, but not epinephrine, stimulates glycogenolysis in the flukes, the possibility was considered that serotonin might increase the activity of phosphorylase in these organisms. In order to prove this hypothesis, phosphorylase activity was determined in cell-free homogenates of flukes which had been cultured with serotonin as well as in control flukes cultured in the absence of this indolalkylamine. It was found that flukes which have been cultured with serotonin, either in the presence or in the absence of glucose, showed higher phosphorylase activity than the controls. The increase in phosphorylase activity produced by serotonin was observed in the presence or absence of adenosinemonophosphate. This has shown that by either assay method phosphorylase activity is increased in flukes cultured with serotonin (2).

Since it was shown by Sutherland and Rall that activation of phosphorylase in cell-free

extracts is related to the concentration of cyclic 3,5-AMP, attempts were made to demonstrate the effects of serotonin on the synthesis of this compound. In collaboration with Drs. Sutherland and Rall, we have been able to demonstrate that particulate preparations from the liver fluke, when incubated with ATP and Mg, formed no significant amount of cyclic 3,5-AMP. However, addition of serotonin (1×10^{-5} M) to the reaction mixture stimulated the synthesis of the nucleotide. Epinephrine, when tested under the same conditions, did not have this effect. When NaF was added to the reaction mixture, serotonin (1×10^{-5} M) also caused a marked increase in the synthesis of the nucleotide, while epinephrine had no effect.

The effects of serotonin, in addition to our previous finding that serotonin is present in these organisms (3), suggest that this compound might stimulate both glycogenolysis and glycolysis. It is possible that serotonin, or a related compound, plays the same role in the carbohydrate metabolism of this invertebrate that epinephrine plays in that of higher organisms.

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DISCUSSION

THE EFFECT OF SYMPATHOMIMETIC AMINES ON PHOSPHORYLASE ACTIVITY OF THE ISOLATED RAT HEART[1]

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From the beautiful work of C. F. Cori, G. Cori, Sutherland and his co-workers, Krebs and Fischer and other investigators in this field we have learned much about the metabolic action of epinephrine (3) and about the role of phosphorylase in the regulation of blood sugar and its possible roles in muscular contraction (1, 2, 3).

We would like to report briefly experiments concerned with the action of epinephrine and other sympathomimetic amines on contraction of the isolated rat heart and on the state of the phosphorylase enzymes in this tissue. We believe that these experiments provide some support for the view discussed by Ellis this morning that at least some of the physiological actions of epinephrine and similar compounds are related to an effect at the early stages of glycogenolysis, or more specifically on the state of the phosphorylase enzymes.

Isolated rat hearts were perfused with Locke solution in a Langendorff apparatus and the contractions recorded on a smoked drum. At the end of an experiment the hearts were quickly frozen in a dry ice-alcohol slush. When drugs with a stimulating effect were administered, the hearts were frozen at the time of maximal response. If no stimulation occurred, the hearts were frozen about one minute after drug administration. Extracts were made from the frozen hearts and phosphorylase activity determined in the presence and absence of adenylic acid as described by Cori and Illingworth (2).

In previous experiments we had demonstrated that in hearts stimulated by the addition

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